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A NEW FLAVONE C-GLYCOSIDE FROM *TRIGONELLA FOENUM GRAECUM*

A. R. SOOD,* B. BOUTARD,† M. CHADENSON,‡ J. CHOPIN‡ and P. LEBRETON†

*Department of Applied Chemistry, Shri Govindram Seksaria, Institute of Technology and Science, Indore 452003 (M.P.), India

† Laboratoires de Phytochimie et de ‡ Chimie biologique, Université Claude Bernard, 69621, Villeurbanne, France

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The seeds of *Trigonella foenumgraecum* L. (Leguminosae, subfamily Lotoideae) are used in medicine and as a spice [1,2]. Different parts of the plant from various countries have been studied for carotenoids [3], coumarins [4], flavonoids [5,6] and steroidal sapogenins [6,7]. The presence of flavone *C*-glycosides, vitexin [8], isovitexin [9] and vicenins [10] has recently been reported in the seeds.

In the present investigation, a new flavone *C*-glycoside has been isolated from the acetone soluble part of the methanol extract of the seeds, in which it appeared as a $FeCl_3$ positive spot of higher R_f value than previously reported [9] for vitexin and isovitexin on silica gel TLC in EtOAc–MeOH–H_2O (100:17:13). Repeated column chromatography on silica gel and crystallization yielded the pure compound as yellow needles, mp 195–198°. It gave characteristic flavonoid colour reactions [11], a brownish–green with $FeCl_3$, yellow with NaOH, and a yellow precipitate with lead acetate. On PC or TLC, it appeared under UV as a dark purple spot, turning bright yellow on exposure to NH_3 or spraying with $AlCl_3$. A positive Molisch test and mobility in 2% AcOH favoured a glycosidic moiety.

The UV spectrum of the compound (main band at 318 nm) suggested the presence of hydroxycinnamyl group and the observed shifts with diagnostic reagents [12] indicated the presence of 5-, 7- and 4′-hydroxyl groups in the flavone skeleton. The IR spectrum showed a strong band of a *p*-disubstituted phenyl ring around 830 cm^{-1}, a carbonyl band of 5-hydroxyflavones around 1650 cm^{-1}, flanked by a shoulder around 1690 cm^{-1} of the cinnamoyl ester group. The compound was acetylated (Ac_2O-pyridine) in the cold and gave colourless needles mp 127–130°. The NMR spectrum (100 MHz, $CDCl_3$) of the acetate showed the presence of three aliphatic ($\delta < 2{\cdot}25$) and four aromatic ($\delta > 2{\cdot}25$) acetate groups, about eight protons between δ 3·5–6 (region of acetylated sugar protons) and eleven protons between δ 6–8·3 (flavone and cinnamate protons), including 2 one-proton singlets at δ 6·42 (flavone H-3) and 6·70 (A-ring proton). These results were in accord with a structure of flavone mono-glycoside monohydroxycinnamate.

Alkaline hydrolysis of the compound gave an ether-soluble cinnamic acid identified as *p*-coumaric acid (UV spectrum and co-chromatography) and a butanol-soluble flavone glycoside which liberated no sugar by acid treatment and showed the UV spectra of apigenin. It was identified as vitexin (8-*C*-glucosylapigenin) by co-chromatography and the chromatographic additional characterization of isovitexin (6-*C*-glucosylapigenin) in the mixture resulting from heating the compound with acid.

The compound is thus a vitexin *p*-coumarate and, since the 5-, 7- and 4′-OH groups have been shown to be free and there is no NMR signal around δ 1·70 (characteristic for 2″-acetoxyl of 8-C-glucosyl flavones [13]), *p*-hydroxycinnamic acid must be linked to 2-OH of the sugar.

Permethylation [14] of the compound gave a MS which showed the expected molecular peak at *m/e* 676 (5%), but the base peak *m/e* 530 and the other main/ peaks corresponded to vitexin heptamethyl ether and its fragments [15], as a result of saponification during permethylation.

To our knowledge, this compound is the first reported vitexin cinnamate, but a closely related product, vitexin 2″-*O*-*p*-hydroxy-benzoate, has been isolated from *Vitex lucens* wood [16].

EXPERIMENTAL

Isolation and purification. The well powdered seeds (5 kg) of *Trigonella foenumgraecum*, purchased from the local market of Indore, Madhya Pradesh, India, were defatted by repeated extraction with light petroleum (60–80°). These were then exhausted with MeOH and the extract was concentrated. This gave a dark brown viscous residue. The residue was extracted with light petrol, $CHCl_3$, CCl_4 and finally extracted exhaustively with Me_2CO. The combined Me_2CO extracts were concentrated and the dark brown residue was subjected to column chromatography on Si gel, the column was eluted with increasing proportions of EtOAc in petrol and finally with pure EtOAc. The first few fractions of the latter gave the flavonoid *A*. This was repurified by column chromatography and was finally recrystallized from EtOAc–MeOH (1:1) to give yellow needles (mp 195–6°); colour reactions on paper: yellow with Benedict's reagent, brown–red with diazotized benzidine + K_2CO_3; R_f values: 0·46 (2% AcOH), 0·56 (15% AcOH), 0·89 (BAW 4:1:5). UV data λ_{max} (nm): MeOH 212, 222 *sh*, 271, 318; $AlCl_3$ 278, 304, 323, 383; $AlCl_3$ + HCl 277, 302, 321, 380; NaOAc 277, 310, 381; NaOAc + H_3BO_3 270, 312; NaOMe 278, 373. MS (*m/e*) after permethylation: 676 (5%, M^+), 530 (100%), 516 (9%), 369 (16%), 355 (95%), 341 (20%).

Acetylation. The glycoside (25 mg) was acetylated (Ac_2O–pyridine) at room temperature for 24 hrs giving a white solid, which was filtered, washed (H_2O) and dried. It was recrystallized from EtOAc–petrol (1:3) and gave colourless needles (mp 127–30°). UV: λ_{max} 273 nm (MeOH).

Alkaline hydrolysis. The glycoside (5 mg) in MeOH (2 ml) was treated by *N* methanolic KOH (2 ml) at room temp. for 30 min. After acidification (2 N HCl) and evaporation, the residue was taken in boiling H_2O and extracted successively with Et_2O and *n*-BuOH. PC of the ether phase showed a fluorescent blue spot: R_f 0·65 (2% AcOH), UV: λ_{max} 300 *sh*, 311 (MeOH), co-chromatographing with *p*-coumaric acid. PC of the BuOH phase showed in UV light a dark spot: R_f 0·15 (2% AcOH), UV data λ_{max} (nm): MeOH 271, 300 *sh*, 336; $AlCl_3$ 276, 281 *sh*, 304, 342, 382; $AlCl_3$ + HCl 276, 281 *sh*,

304, 342, 381; NaOAc 280, 304 *sh*, 364; NaOH 280, 329 *sh*, 400. The eluted compound co-chromatographed with vitexin and, on heating for 3 hr with 4 N HCl–MeOH (1:1), gave a mixture of vitexin and isovitexin which co-chromatographed with authentic samples.

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OCCURRENCE OF 2-METHYLISOFLAVONES IN *GLYCYRRHIZA GLABRA*

DEVENDRA K. BHARADWAJ, RAMASWAMY MURARI, TIRUVENKATA R. SESHADRI and RADHIKA SINGH

Department of Chemistry, University of Delhi, Delhi-110 007, India

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A number of flavonoids have been reported from time to time from the roots of *Glycyrrhiza glabra*. Samples examined in different countries seem to vary in composition. Recently, the plant has been cultivated in India for the first time. The Indian grown *Glycyrrhiza glabra* has not been studied in detail before. The sample used in the present work has been obtained from the experimental gardens of Govt. Ayurvedic College, Jammu, kindly supplied by Dr. S. C. Sankhyadhar.

The air dried roots have yielded as ether soluble components 3 new compounds. The air dried powdered roots (1·2 kg) were extracted with hot EtOH and the extract concentrated. The solvent-free residue was repeatedly extracted with Et_2O and the combined Et_2O extract was evaporated and the residue chromatographed over Si gel. C_6H_6–EtOAc (3:1) eluted compounds A–C which were further purified by preparative TLC and crystallisation from light petrol. In other fractions quercetin, kaempferol, apigenin, liquiritigenin and isoliquiritigenin were also identified, confirming that the roots are liquorice.

Compound A. (20 mg), mp 161–162°, gave −ve $FeCl_3$, −ve Mg–HCl, −ve Zn–HCl but +ve Na–Hg–HCl tests. λ_{max}^{MeOH} 230, 295 nm (no shift with $AlCl_3$, NaOAc or NaOMe). ν_{max}^{KBr} 1750, 1640, 1245 cm^{-1}. The colour reactions and UV spectrum indicated A to be an isoflavone.

MS. 294 (M^+), 252, 137, 136, 77. *NMR* (TMS internal standard) (δ, $CDCl_3$): 2·30, 2·40 (6H, OCOMe and Me), 7·20, 7·35 (*dd*, 2H, 6-H, 8-H, J_m 2 Hz, J_o 8 Hz; the *m* coupled signal of 8-H is superposed over the signal at δ 7·35), 7·50 (*m*, 5H side phenyl protons), 8·50 (*d*, 1H, *J* 8 Hz, 5-H). The IR and NMR indicated the presence of an acetoxyl function in the compound. Hydrolysis of A with methanolic HCl gave a phenol identical with compound C. The NMR spectrum of A further indicated the presence of (i) mono-substituted phenyl system, (ii) unsubstituted resacetophenone system and (iii) an aromatic methyl or a methyl attached to a C–C double bond. Mass spectrum also supported the conclusions (i) and (ii). Further, alkali hydrolysis of A gave phenylacetic acid. Thus the possibilities of the *C*-methyl being in the rings A or B of the isoflavone unit are eliminated. Hence it must be in the pyrone ring; thus, compound A is 7-acetoxy-2-methylisoflavone. The above structure was confirmed by comparison with an authentic sample prepared